Mass Spectrometry Contamination from Tinuvin 770, a Common Additive in Laboratory Plastics

Kevin L. Schauer,[1] Carolyn J. Broccardo,[1] Kimberly M. Webb,[2] Paul A. Covey,[2] and Jessica E. Prenni[1,3]

[1]Colorado State University Proteomics and Metabolomics Facility, Fort Collins, Colorado, USA; [2]U.S. Department of Agriculture-Agricultural Research Service, Sugarbeet Research Unit, Fort Collins, Colorado, USA; and [3]Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, Colorado, USA

The superior sensitivity of current mass spectrometers makes them prone to contamination issues, which can have deleterious effects on sample analysis. Here, bis(2,2,6,6-tetramethyl-4-piperidyl) sebacate (marketed under the name Tinuvin 770) is identified as a major contaminant in applications using liquid chromatography coupled with mass spectrometry (LC-MS). Tinuvin 770 is often added to laboratory and medical plastics as a UV stabilizer. One particular lot of microcentrifuge tubes was found to have an excess of this compound that would leach into samples and drastically interfere with LC-MS data acquisition. Further analysis found that Tinuvin 770 readily leached into polar and nonpolar solvents from the contaminated tube lot. Efforts to remove Tinuvin 770 from contaminated samples were unsuccessful. A prescreening method using MALDI-TOF MS is presented to prevent system contamination and sample loss.

Key Words: bis(2,2,6,6-tetramethyl-4-piperidyl) sebacate, liquid chromatography, proteomics, microcentrifuge tubes, LC-MS

INTRODUCTION

Liquid chromatography coupled with mass spectrometry (LC-MS) is a valuable analytical tool routinely used for the analysis of biological molecules (e.g., proteins, peptides, and metabolites), as well as in other fields. The high sensitivity of current MS technologies allows for superior levels of detection, even within a complex matrix. For example, instruments used in proteomic studies are routinely capable of detecting low femtomole levels of a peptide.[1] However, the high sensitivity of these instruments also makes them prone to contamination. As an example, in proteomics experiments, keratin is often introduced during sample-handling steps. As many mass spectrometers use data-dependent acquisition techniques, such contamination can lead to considerable data loss, as MS time is dominated by the analysis of keratin peptides instead of the peptides of interest.[2,3] Nonproteinaceous materials can also have deleterious effects on data collection in LC-MS experiments.[4] For example, high salt concentrations can inhibit ionization, and certain plasticizers have also been shown to negatively affect data acquisition through ion suppression.[4]

Address correspondence to: Jessica E. Prenni, Colorado State University, 2021 Campus Delivery, Fort Collins, CO 80523, USA (Phone: 970-491-0961; E-mail: jessica.prenni@colostate.edu).
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Here, bis(2,2,6,6-tetramethyl-4-piperidyl) sebacate, commonly known as Tinuvin 770, was detected as a major contaminant in a LC-MS/MS proteomics experiment. This compound is a UV stabilizer,[5] commonly used in the production of plastics, such as polypropylene and polystyrene.[6] Previous studies have shown that Tinuvin 770 can leach from polypropylene tubes and interfere with common laboratory procedures, as well as have toxic effects on laboratory animals.[6–9] However, this compound has not, until now, been shown as a potential contaminate in LC-MS or LC-MS/MS applications.

MATERIALS AND METHODS

LC-MS

Protein extracts from *Beta vulgaris* leaves were subjected to tryptic digestion, as described previously.[10] In brief, 30 μg protein was precipitated in acetone and resuspended in 8 M urea with 0.2% ProteaseMAX surfactant (Promega, Madison, WI, USA) by bath sonication. Proteins were then reduced using DTT and alkylated by iodoacetamide. Trypsin digestion was performed at 37°C for 3 h and was stopped by the addition of trifluoroacetic acid to a final concentration of 0.5%. Digested peptides were dried in a vacuum evaporator and purified using a reverse phase C18 TopTip (Glygen, Columbia, MD, USA). Purified peptides

were reconstituted in 30 μl 0.1% formic acid with 3% acetonitrile (ACN) in preparation for LC-MS analysis.

Peptides were separated temporally on a reverse phase nanospray column (1200 Nano HPLC and Zorbax C18, 5 μm, 75 μm inner diameter×150-mm column; Agilent Technologies, Santa Clara, CA, USA), using a 90-min linear gradient from 25% to 55% buffer B (90% ACN, 0.1% formic acid) at a flow rate of 300 nl/min. Eluted peptides were injected directly into a linear ion trap mass spectrometer (LTQ; Thermo Fisher Scientific, Waltham, MA, USA), where spectra were collected over a mass window of 200–2000 mass-to-charge ratio (m/z). A dynamic exclusion limit of two MS/MS spectra for a given m/z in a 30-s period (followed by a 90-s exclusion of that ion) was used.

MALDI-TOF/TOF MS

One microliter of the contaminated sample was co-spotted with 1 μl of 10 mg/ml α-cyano-4-hydroxy-cinnamic acid (CHCA) matrix (Bruker Daltonics, Bremen, Germany) in 50% ACN and 0.1% trifluoroacetic acid onto a MTP 384 ground steel MALDI target plate (Bruker Daltonics). This mixture was allowed to dry at room temperature and then analyzed using an Ultraflex II MALDI TOF/TOF (Bruker Daltonics). Acquisition was performed in positive ion reflector mode with a 25-kV acceleration voltage. External calibration was performed using the Bruker peptide calibration standard II that had been spiked with 0.1 μg/μl estrone and progesterone (Sigma-Aldrich, St. Louis, MO, USA) to extend the range of the calibration. In addition, the CHCA matrix was analyzed under identical conditions to determine which of the observed peaks in the sample spectra were a result of matrix background.

Abundant, nonmatrix peaks observed in the MS spectrum were then subjected to MS/MS analysis using the same instrumentation in LIFT mode. Each parent mass was searched against the MassBank mass spectral database (http://www.massbank.jp/?lang=en), and the corresponding experimental MS/MS spectra were manually compared with the database spectra. The authentic, standard Tinuvin 770 (Sigma-Aldrich; solution in methanol) was also analyzed using the procedure described above.

Tube-Contamination Analysis

Microcentrifuge tubes were obtained from five different vendors: Daigger (Vernon Hill, IL, USA), ExtraGene (Taichung City, Taiwan), Quasar Instruments (Colorado Springs, CO, USA), Light Labs (Dallas, TX, USA), and Life Science Products (Frederick, CO, USA). The lot numbers and manufacturers of the tubes are shown in Table 1. A minimum of five tubes from each lot was tested, each using a different solvent, including 100% methanol, 0.1% formic acid, 50 mM ammonium bicarbonate (Sigma-Aldrich), 100% acetone and 100% chloroform (Fisher Scientific, Fair Lawn, NJ, USA). For each test, a volume of 100 μl solvent was added to the tube, vortexed briefly, and incubated at room temperature for 20 min. The test sample was then spotted onto the MALDI target, along with the CHCA matrix, as described above. Mass spectra were acquired using the same instrumentation and settings described previously with the following exceptions: only MS data were collected, and the firing location was automated to eliminate sampling bias as a result of inhomogeneous matrix crystallization. Laser power was held constant for the analysis of all test samples. Automation was configured such that a total of 800 shots was collected in eight different positions within each test sample spot.

RESULTS

Sample contamination was first realized while analyzing a complex peptide mixture using LC-MS/MS as described above. Approximately 20 min into the gradient, a large peak was observed that was abnormal for the sample type (Fig. 1A and B). The MS spectra revealed an intense doubly charged ion with a m/z of 241, along with two much less-intense ions, at 342 and 482 m/z (Fig. 1C). The presence of this contaminant had a dramatic, deleterious

TABLE 1

Summary of the Tubes Tested for the Presence of Tinuvin 770

Retailer	Catalog number	Manufacturer	Lot number	Tinuvin 770 present?
Daigger	EF8978A	ExtraGene	3124522321	Yes
ExtraGene	TUBE-170-C	ExtraGene	56171204260	No
Quasar Instruments	5024-TUBE-170-C	ExtraGene	312452121	No
Quasar Instruments	5024-TUBE-170-C	ExtraGene	104571010	No
Quasar Instruments	5024-TUBE-170-C	ExtraGene	900117188	No
Quasar Instruments	5024-TUBE-170-C	ExtraGene	530494070	No
Light Labs	A-7011	Scientific Specialties Service	12113	No
Life Science Products	M-1700C	Sorenson BioScience	V22909	No

Scientific Specialties Service (Hannover, MD, USA); Sorenson BioScience (Salt Lake City, UT, USA).

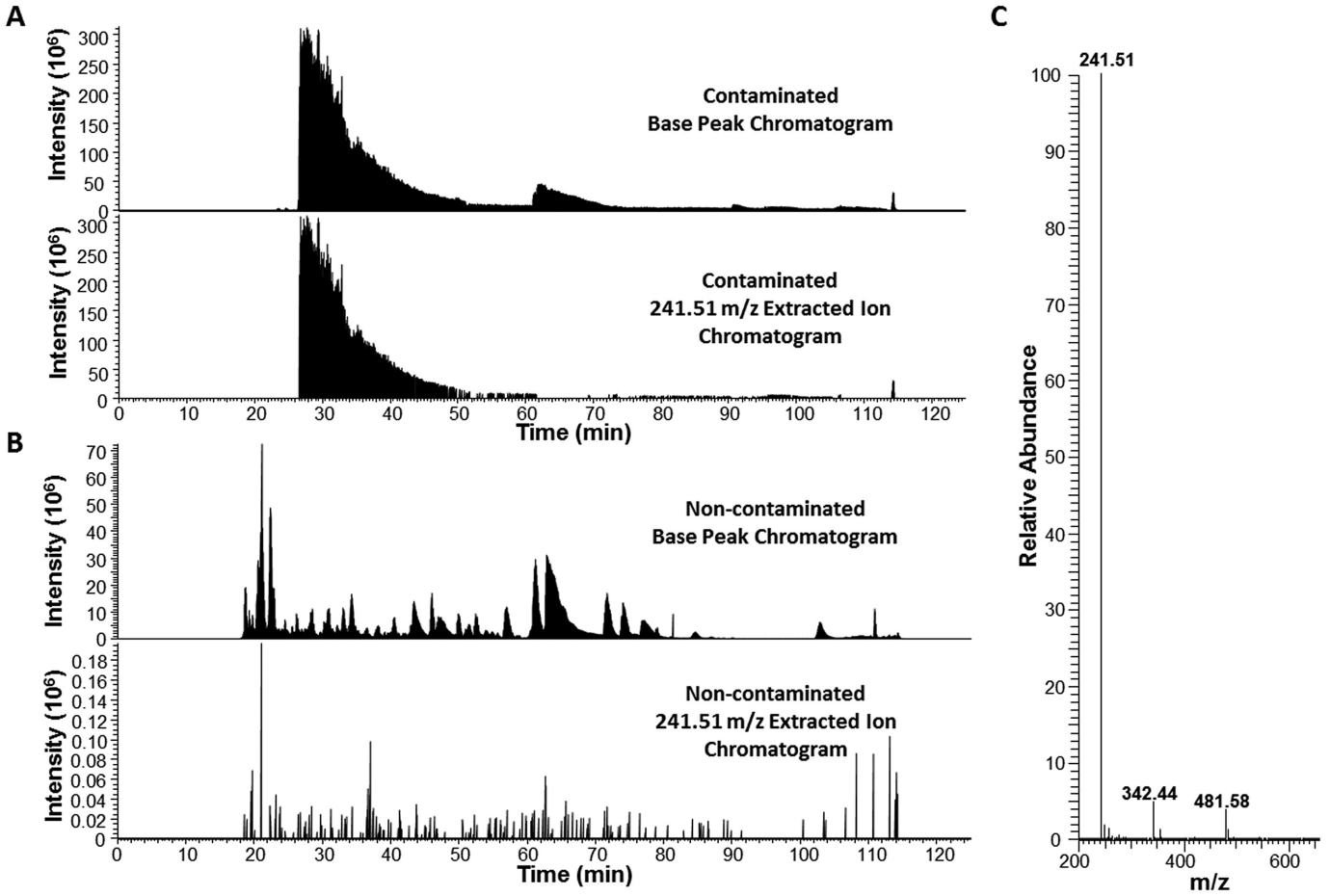

FIGURE 1

(A) Base peak and 241.51 m/z-extracted ion chromatograms for a contaminated sample and (B) a sample lacking the contaminant. (C) Full MS scan spectrum during the elution of 241.51 m/z.

effect on the number of proteins identified in the experiment as a result of ion suppression of coeluting peptides. In addition, the same ions were present in subsequent injections of different samples, suggesting instrument contamination. Only with extensive flushing (>12 h) in high organic solvent (ACN) was the contaminant removed completely.

To determine the source of the observed contamination, an aliquot of the contaminated sample was subjected to MALDI-MS analysis, as described in Materials and Methods. The doubly charged peak, observed previously by LC-MS (241 m/z), was not observed; however, an intense peak at 481.5 m/z was observed, representing the singly charged state of the same compound (Fig. 2A). This ion was subjected to MS/MS analysis, and the parent mass was searched against the MassBank spectral database. Manual comparison of the database spectra and the MALDI-MS/MS spectrum collected from the contaminated sample led to the putative identification of Tinuvin 770. The identity of the contaminant was subsequently confirmed by MALDI-MS/MS analysis of an authentic Tinuvin 770 standard under the same conditions (Fig. 2B).

In an effort to find the source of the observed contamination, a microcentrifuge tube from the same package as the original was tested for the presence of Tinuvin 770, along with the pipette tips that were used during the sample preparation. There was no evidence of Tinuvin 770 being leached from the tips (data not shown); however, when a small volume of methanol was incubated in the tube and then analyzed by MALDI-MS/MS, a spectral match for Tinuvin 770 was observed. To determine whether the observed contamination issue was large-scale or an isolated incident, microcentrifuge tubes from a variety of retailers and manufacturers were obtained and tested for the presence of Tinuvin 770. Specifically, the tubes tested included (Table 1): five lots of tubes identical to the original contaminated samples with one lot being obtained directly from the manufacturer (ExtraGene) and the remaining four lots from a local distributor (Quasar Instruments), as well as comparable tubes from several other manufacturers

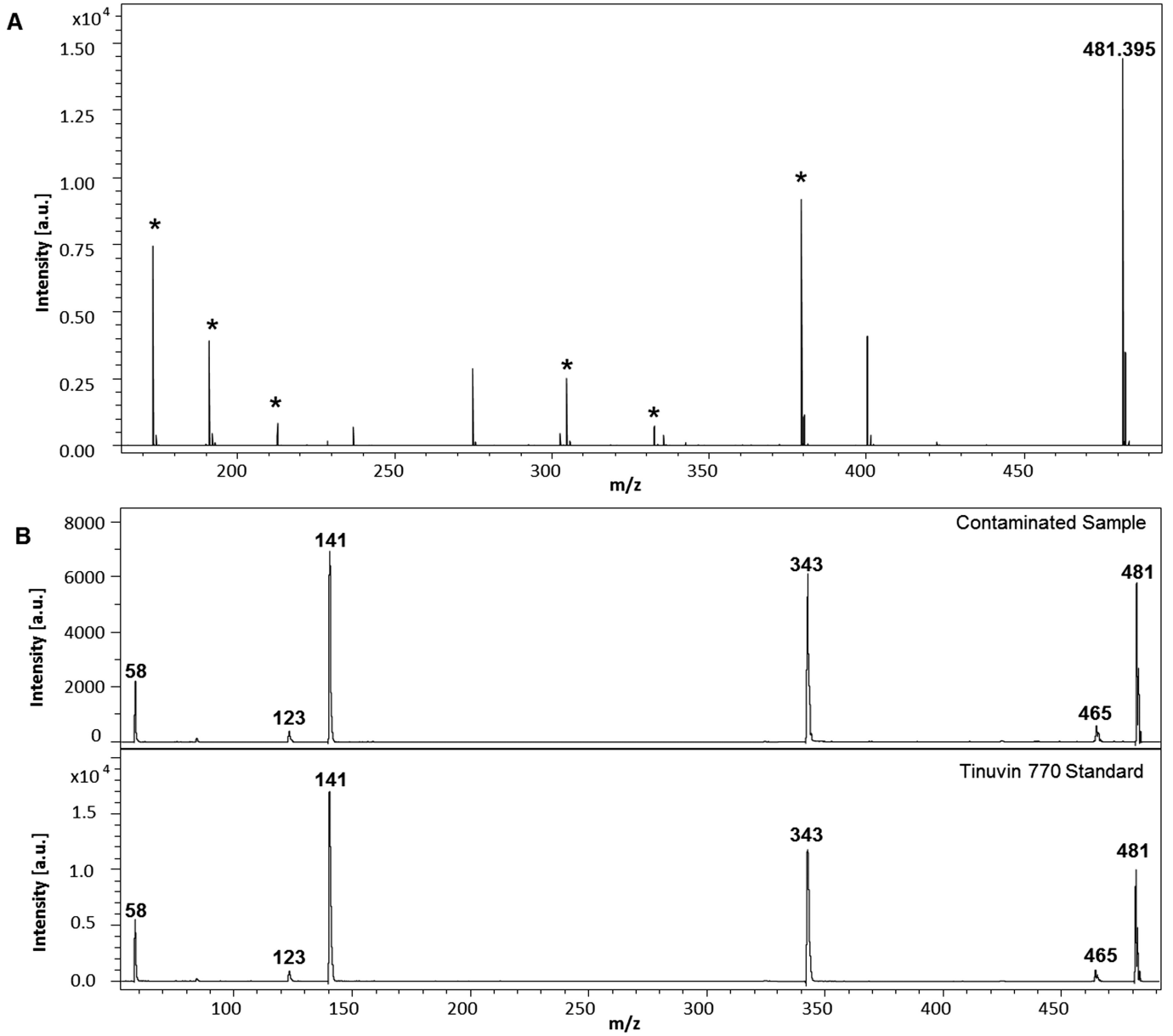

FIGURE 2

MALDI-MS data used to identify the contaminant in the sample. (A) MALDI-MS spectrum showing the prominent 481 peak. Peaks labeled with an asterisk are known background from the CHCA matrix. (B) Side-by-side comparison of the MALDI-MS/MS spectra collected from the contaminated sample and the Tinuvin 770 standard using a parent mass of 481 m/z.

and suppliers. Of all the tubes included in the study, only those from the original contaminated lot (ExtraGene) showed spectral evidence of Tinuvin 770, and this was observed with all solvents tested. Once the contaminant was identified, several commercially available and commonly used clean-up procedures were investigated for their ability to remove it. The peptide samples where the Tinuvin 770 contamination was discovered initially had already undergone a reverse-phase cleanup procedure using Pierce C18 spin columns (Thermo Fisher Scientific, Rockford, IL, USA) as part of the protein digestion procedure (described above). In another attempt to remove the contaminant from the samples, TopTip polysulfoethyl A (Glygen) strong cation exchange columns were used in accordance with the manufacturer's instructions. MALDI analysis of pre-cleanup and post-cleanup samples showed little to no decrease in Tinuvin 770 abundance (data not shown).

DISCUSSION

Here, we have shown that Tinuvin 770 can leach from standard polypropylene microcentrifuge tubes and thus, interfere with LC-MS applications. The extent of contam-

ination is illustrated in an example proteomics experiment, although ion suppression at this level would be detrimental to any LC-MS application. Whereas contamination was only detected in one lot of tubes from one manufacturer, it is likely that the contamination could occur in future lots, from other manufactures, or from plasticware that was not tested in this study. As a result of the inability to remove Tinuvin 770 by standard cleanup protocols, samples that have been placed in a contaminated tube are rendered useless because of the significant levels of ion suppression. In addition, LC-MS instruments that become contaminated with this compound require extensive cleaning, which can lead to extensive down-time. Thus, the impacts of even a small number of contaminated tubes could be far-reaching and costly. Further work is required to develop an effective Tinuvin 770 removal procedure that is compatible with LC-MS workflows. Until then, prescreening by MALDI-TOF, as described above, prior to LC-MS analysis can be used to avoid system contamination and data loss.

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